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A new flavonoid derivative as cytotoxic compound isolated from ethyl acetate extract of *Macaranga gigantifolia* Merr. Leaves

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Abstract

A new prenylated flavonoid compound 5,7,3',4'-tetrahydroxy-3,6-diprenylflavone (1) has been isolated from methanol extract of the leaves of *Macaranga gigantifolia* Merr. Isolation was performed using chromatography methods and their structures were elucidated based on spectroscopic data. cytotoxic activity against murine leukemia P-388 cell line conducted using MTT method. Compound 1 showed strong cytotoxic activity with IC_{50} value 6.19 µg/mL

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Keywords: Macaranga gigantifolia; Euphorbiaceae; 5,7,3',4'-tetrahydroxy-3,6-diprenylflavon; murine leukemia P-388 cell line.

1. Introduction

Genus Macaranga (Euphorbiaceae) comprises more than 308 species and wide spread from Afrika and Western Madagascar to Asia tropical regions (including Indonesia), North Australia and eastern pacific islands1. In Indonesia, this genus known as "mahang" has been part of the traditional medicine system to treat diarrhea, wound and cough[2]. Our previous study showed that *Macaranga* has potency as antioxidant[3-8], anticancer[8-13], antibacterial[3,14], insecticidal[16], antidiabetic[17], and anti-implammation[18]. *Macaranga gigantifolia* Merr. is one of the *Macaranga* species, distributed mainly in Sumatera, Kalimantan and Maluku[19], used traditionally to treat diarrhea and thrush[20]. Previous study showed that scopoletin, a secondary metabolite isolated from *M. gigantifolia* has anticancer activity against murine leukemia P-388 cell lines with IC_{50} 17.42 µg/ml[12]. Biological activities potential,

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especially in cytotoxicity activity of the secondary metabolite compounds isolated from the other *Macaranga* species lead us to carry out further phytochemical studies on *M. gigantifolia*. In continuation of our research about anticancer active compounds from *Macaranga* plants, we report herein the isolation of a new prenylated flavonoid compound, 5,7,3',4'-tetrahydroxy-3,6-diprenylflavone (1), and its cytotoxicity activity against murine leukemia P-388 cells.

2. Material and Methods

2.1 General

^{1}H-and 13C-NMR spectra were recorded with a JEOL JMN-ECA 500 Spectrometer. Vacuum liquid chromatography (VLC), centrifugal planar chromatography and TLC analysis were carried out using silica gel 60 G (1.07731.1000, Merck KGaA), silica gel 60 PF254 (1.07749.1000, Merck KGaA) and pre-coated silica gel 60 PF254 plates (1.05554.0001, Merck KGaA), respectively. Extraction, fractionation and separation using distilled technical grade solvent.

2.2 Plant material

The leaves of Macaranga gigantifolia Merr. were collected in March 2012 from District Kolaka, Southeast Sulawesi, Indonesia, and identified at Herbarium Bogoriense, Research Center for Biology, Indonesian Institute of Sciences, Bogor, Indonesia, where a voucher specimen were deposited.

2.3 Extraction and isolation

Powdered dried leaves of M. gigantifolia (2 kg) were macerated in methanol for three times (3 x 10L). Methanol extract was evaporated to obtain 351 g of crude methanol extract. Methanol crude extract (114 g) was fractionated using silica gel VLC with n-hexane, ethyl acetate and acetone as mobile phase. Ethyl acetate fraction subjected to silica gel column chromatography using gradient system solvent of n-hexane, ethyl acetate and methanol to give five major fractions F1-F5. F1 and F5 further chromatographed using centrifugal planar chromatography eluted with n-hexane-EtOAc (6:4→ 1:9), EtOAc, EtOAc-MeOH (9:1→7:3) to obtain compound 1 (6 mg).

2.4 Cytotoxic evaluation

Cytotoxic properties performed using MTT assay method based on21-22. Living cells 3 x 103/mL were plated in 96-well culture dishes. Plate was incubated for 24 hours in humidified CO2 incubator 37°C. After incubation, 10 µL medium containing various concentration of the sample were added. After 48 hours incubation, medium was removed from the well plate and 150 µL of fresh medium + 50 µL MTT reagent was added. Incubation continued for next 4 hours. After 4 hours incubation, MTT removed and insoluble formazan dissolved in 50 µL DMSO. Optical density measured on microplate reader at 550 nm. IC_{50} calculated according to Cricket Programs.

3. Result and Discussion

From the leaves of Macaranga gigantifolia Merr. collected from Mekongga forest, District Kolaka, Southeast Sulawesi, Indonesia, a new compound 5,7,3',4'-tetrahydroxy-3,6-diprenylflavone (1) was isolated (Fig. 1b). Structure elucidation of compound 1 performed based on FT-IR, ESI-MS/HR-ESI-MS and FT-NMR techniques.

Compound 1, yellow powder, HR-ESI/MS m/z [M-H]- 421.1635 (calcd. for C25H25O6, 421.1631). The 1H-NMR spectrum of compound 1 (Table I) showed one hydrogen bridge at δH 12.43 (s, 1H, -OH), three aromatic proton signals for ABX proton system at δH 8.14 (1H, d, J = 8.43 Hz); 7.97 (1H, dd, J = 8.43 and 1.95 Hz) and 8.05 ppm (1H, d, J = 1.95 Hz) corresponding to the group substituent at C-3' and C-4' in ring B flavon. A proton singlet signal at δH 6.58 ppm showed a different substituent bonded to ring A of flavon structure. Two prenyl substituent of compound 1 characterized by the presence of two vinyl groups at δH 5.28 (1H, t, J=7.14 Hz) and 5.38 ppm (1H, t, J= 7.14 Hz), supported with four singlet peaks of methyl group at δH 1.65 (3H, s); 1.75 (3H, s); 1.751 (3H, s) and 1.78 ppm (3H, s), and two methylene group peaks at δH 3.36 (2H, d, J= 7.14 Hz) and 3.39 ppm (2H, d, J= 7.14 Hz). 13C-NMR spectrum showed 25 carbon signals. Proton signal at δH 3.39 (2H, d, J= 7.14 Hz)(H-1") which has correlation in HMBC with δC 129.1 ppm (C-3), carbonyl signal at δC 176.6 ppm (C-4) and with quaternary carbon atom δC 157.8 ppm (C-2) indicated that of the prenyl constituent of compound 1 bonded to C-3 at ring B. Other HMBC spectra showed correlation between methylene signal at δH 3.36 ppm (2H, d, J = 7.14 Hz) with quaternary

carbon at δC 111.7 (C-6), and two oxyaril signals at δC 158.9 (C-5) and 162.7 (C-7) indicated another prenyl substituent attached to C-6 (Fig. 1a). Compound 1 also have six oxyaril carbon signals at δC 147.0 (C-4'); 155.6 (C-8a); 157.8 (C-2); 158.9 (C-5); 160.2 (C-3') and 162.7 ppm (C-7).

Table I. NMR data of compound 1 in acetone-d6

No.	δ_H (ΣH, multiplicity, *J* in Hz)	δ_C	HMBC (H↔C)
2	-	157.8	
3	-	129.1	
4	-	176.6	
4a		104.1	
5	-	158.9	
6	-	111.7	
7	-	162.7	
8	6.58 (1H, *s*)	93.8	C-4a, C-6, C-7, C-8a
8a	-	155.6	
1'	-	123.5	
2'/6'	8.05 (1H, *d*, 1.95)	130.3	C-2, C-4'
3'	-	160.2	
4'	-	147.0	
5'	8.14 (1H, *d*, 8.43)	130.5	C-3', C-4', C-6'
6'	7.97 (1H, *dd*, 8.43)	128.0	C-2, C-2', C-4'
1"	3.39 (2H, *d*, 7.14)	29.1	C-2, C-3, C-2", C-3"
2"	5.28 (1H, *t*, 7.14)	123.3	C-4", C-5"
3"	-	133.2	
4"	1.75 (3H, *s*)	17.9	
5"	1.75 (3H, *s*)	25.9	
1'"	3.36 (2H, *d*, 7.14)	22.0	C-5, C-6, C-7, C-2"', C-3'"
2'"	5.38 (1H, *t*, 7.14)	123.2	C-3'", C-4'", C-5'"
3'"	-	131.7	
4'"	1.78 (3H, *s*)	17.9	
5'"	1.65 (3H, *s*)	25.9	

Based on 1D- and 2D-NMR, supported by HR-ESI/MS data and literature survey, compound 1 was identified as a new flavonoid compound named 5,7,3'4'-tetrahydroxy-3,6-diprenylflavon (Fig. 1b).

Compound 1 isolated from the leaves of M. gigantifolia was evaluated for their cytotoxicity activity against murine leukemia P-388 cells. The result showed that compound 1 has strong cytotoxic activity with IC50 value 6.19 μg/mL. Two prenyl groups attached to flavonoid increase lipophilicity feature of the compound, it was correlated to the ability of the compound to penetrate the wall of the cancer cell. Based on the cytotoxic data above indicated that prenyl group substituent increases the anticancer activity. Further cytotoxic testing of the flavonoid derivatives prenylated are required to ascertain the effect of the prenyl group substituent on cytotoxic activity.

Based on our best knowledge, 5,6,3',4'-tetrahydroxy-3,6-diprenylflavon (1) is a new prenylated flavonoid compound isolated and also reported for the first time on M. gigantifolia plant.

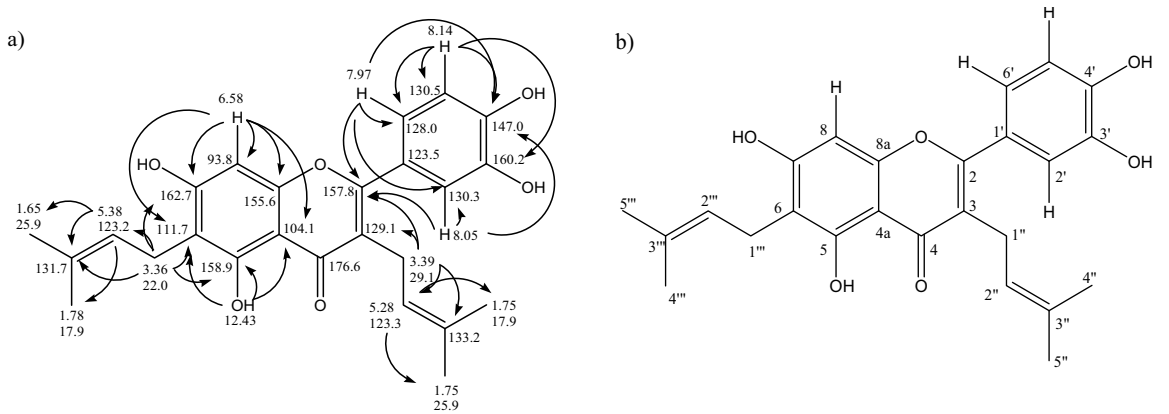

Fig. 1. (a) HMQC- and HMBC-NMR correlations, (b) chemical structure of 5,7,3’4’-tetrahydroxy-3,6-diprenylflavon

4. Conclusion

A new flavonoid compound 5,6,3’,4’-tetrahydroxy-3,6-diprenylflavon (1), has been isolated from the leaves of Macaranga gigantifolia Merr. Cytotoxic properties of compound 1 showed that 5,6,3’,4’-tetrahydroxy-3,6-diprenylflavon (1) has a strong cytotoxic activity against murine leukemia P-388 cells.

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References

1. Blattner FR, Weising K, Banfer G, Maschwitz U, Fiala B. Molecular analysis of phylogenetic relationships among myrmecophytic Macaranga species (Euphorbiaceae). Mol Phyl Evol 2001;19:331-4.
2. Heyne K. Useful Plants of Indonesia (in Indonesian), 1st ed. Jakarta: Yayasan Sarana Wanajaya; 1987.
3. Jang DS, Cuendet M, Hawthorne ME, Kardono LBS, Kawanishi K, Fong HHS, Mehta RG, Pezzuto JM, Kinghorn AD. Prenylated flavonoids of the leaves of Macaranga conifera with inhibitory activity against cyclooxygenase-2. Phytochemistry 2002;61:867-73.
4. Phommart S, Sutthivaiyakit P, Chimnoi N, Ruchirawat S, Sutthivaiyakit S. Constituents of the leaves of Macaranga tanarius, J Nat Prod 2005;68:927-30.
5. Sutthivaiyakit S, Unganont S, Sutthivaiyakit P, Suksamrarn A. Diterpenylated and prenylated flavonoids from Macaranga denticulata. Tetrahedron 2002;58:3619-22.
6. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti tyrosinase activities of four Macaranga species. Food Chem 2009;114:594-9.
7. Kumazawa S, Murase M, Momose N, Fukumoto S. Analysis of antioxidant prenylflavonoids in different parts of Macaranga tanarius, the plant of Okinawan propolis. Asian Pasific Journal of Tropical Medicine 2014:7(1):16-20.
8. Aminah NS, Kristanti AN, Tanjung M. Antioxidant activity of flavonoid compounds from the leaves of Macaranga gigantea. J of Chem and Pharm Res 2014;6(6):688-92.
9. Beutler JA, Shoemaker RH, Johnson T, Boyd MR. Cytotoxic geranyl stilbenes from Macaranga schweinfurthii. J Nat Prod 1998;61:1509-12.
10. Beutler JA, Jato J, Cragg GM, Boyd MR. Schweinfurthin D. A cytotoxic stilbene from Macaranga schweinfurthii. Nat Prod Lett 2000;14:399-404.
11. Li X, Xu L, Wu P, Xie H, Huang Z, Ye W. Prenylflavonols from the leaves of Macaranga sampsonii. Chem Pharm Bull 2009;57:495-8.
12. Darmawan A, Kosela S, Kardono LBS, Syah YM. Scopoletin, a coumarin derivative compound isolated from Macaranga gigantifolia. J of App Pharm Sci 2012;2(12):175-7.
13. Zakaria I, Ahmat N, Jaafar FM, Widyawaruyanti A. Flavonoids with antiplasmodial and cytotoxic activities of Macaranga triloba. Fitoterapia 2012;83:968-72.

14. Schutz BA, Wright AD, Rali T, Sticher O. Prenylated flavanones from the leaves of Macaranga pleiostemona. Phytochemistry 1995;40: 1273-7.
15. Phommart S, Sutthivaiyakit P, Chimnoi N, Ruchirawat S, Sutthivaiyakit S. Constituents of the leaves of Macaranga tanarius, J Nat Prod 2005;68:927-30.
16. Rahman SS, Rahman MM, Khan MMR, Begum SS, Roy B, Shahed SMF. Ethanolic extract of melgota (Macaranga postulata) for repellency, insecticidal activity against rice weevil (Sitophilus oryzae). African J of Biotech 2007;6(4):379-83.
17. Gunawan-Puteri MDPT, Kawabata J. Novel α-glucosidase inhibitors from Macaranga tanarius leaves. Food Chemistry 2010;123:384-9.
18. Phommart S, Sutthivaiyakit P, Chimnoi N, Ruchirawat S, Sutthivaiyakit S. Constituents of the leaves of Macaranga tanarius, J Nat Prod 2005;68:927-30.
19. Whitmore TC, Tantra IGM, Sutisna U. Tree Flora of Indonesia: Check List for Sulawesi. Jakarta: Indonesia Ministry of Forestry, Agency for Forestry Research and Development, Forest Research and Development Centre; 1989.
20. Kulip J. Similarity of medicinal plants used by two native communities in Sabah, Malaysia Proc. WOCMAP III. Vol. 1: Bioprospecting & Ethnopharmacology Eds. J. Bernáth, É. Németh, L.E. Craker and Z.E.Gardner. Acta Hort 2005;675, ISHS 2005.
21. Tanjung M, Mujahidin D, Juliawaty LD, Makmur L, Achmad SA, Hakim EH, Syah YM. Flavonoids from Macaranga gigantea (Euphorbiaceae). Proceeding of the International Seminar on Chemistry. Padjajaran University, Jatinangor Indonesia. 30-31 October 2008. ISBN 978-979-18962-0-7, 252-255.
22. Harneti D, Tjokronegoro R, Safari A, Supratman U, Xe-Min L, Mukhtar MR, Mohamad K, Awang K, Hayashi H. Cytotoxic triterpenoid from the bark of Aglaia smithii (Meliaceae). Phytochemistry Letters 2012;5:496-9.